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A RAPID METHOD OF MAKING NUTRIENT AGAR-AGAR.

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The principal difficulty in the preparation of nutrient agar-agar has generally been the slowness with which the solution passes through the filter. Various devices, relating partly to the method of mixing or boiling the ingredients and partly to the character of the filter used, have been employed to overcome this difficulty. Hot water filters of one form or another are generally considered necessary.

The following method of preparing nutrient agar-agar has for some time been in use in the Pathological Laboratory of the Johns Hopkins University and Hospital. As it has given great satisfaction and appears to be simpler and more rapid than any hitherto suggested, it may be useful to publish its details here. The entire process up to the filling of the test tubes can be accomplished within one hour.

The boiling is done over a free flame in an enameled iron vessel, a so-called agate saucepan, of a capacity of two litres. The heat is furnished by a gas stove with multiple gas jets arranged in a circle.

To 1500 cc. of water (which need not be distilled) add 18 grams of agar-agar and boil *vigorously* for thirty minutes. No cover is placed over the vessel and stirring of the liquid is not necessary. During this time a thick, white scum appears floating on the surface; this should be removed. 2 grams of Liebig's Beef Extract are added while the mixture is boiling. At the end of thirty minutes the vessel is removed from the fire and the whole allowed to cool to 60° C. 10 grams of dry peptone (Witte's) and 5 grams of sodium chloride (C. P.) are now added, and the entire contents of an egg in water (the quantity of water depending upon that previously evaporated) are thoroughly mixed with the agar solution. The reaction is now tested and is generally found to be too strongly alkaline. By the careful addition of dilute HCl a very faintly alkaline or neutral reaction can be obtained, or preferably this neutralization may be made before adding the egg. The saucepan containing the mixture is now put over the flame again and is boiled for five to ten minutes. The mixture is now to be filtered through the best white filter paper[1] previously moistened with water. No

[1] There are differences in filter paper regarding the rapidity with which filtration is effected. We have found several sorts of soft white filter paper of good quality to answer the purpose.

hot water funnel is needed. The mixture filters rapidly, and can be run through the same filter a second time if desired. If the filtered solution is not fairly clear and transparent, it may be improved by the addition of the white of another egg, the solution being afterward cooked merely long enough to coagulate the albumen of the egg. At the end of the process one should have 1000 cc. of the medium.

Each step in this process is important. A litre will readily filter through clear in from 3 to 5 minutes, this rapid filtration being secured by the coarse character of the precipitate and the consistence of the solution. With a little experience one can and should always judge beforehand by the clearness or transparency and the thin consistence of the fluid whether it is in the right condition for filtration. If the fluid has not these properties the reaction is doubtless too strongly alkaline.

Nutrient agar thus prepared can be used at once for Esmarch roll tubes, even after the addition of 4 per cent glycerine, which is the most severe test of the proper consistence of the medium.

If it is desired to prepare the medium with infusion of meat instead of with beef extract, then the procedure is modified as follows: One pound of finely cut lean meat is digested in 1500 cc. of water at a temperature of 50° C. for thirty minutes, is strained through a linen cloth or towel, is boiled five minutes and is then filtered. To this filtrate in an agate saucepan agar-agar is added and the subsequent process is the same as that already described. The reaction of the agar-agar solution made with this meat infusion is of course decidedly acid, and a neutral or alkaline reaction requires the addition of a saturated solution of sodium carbonate until the desired reaction is obtained.

As has already been mentioned, the whole time required for making nutrient agar-agar with Liebig's beef extract by this method is not more than an hour. No hot water funnel is required, filtration of a litre taking place within three or four minutes. The consistence of the medium is at once suitable for plate and roll cultures.

We cannot agree with all of the recommendations of Dr. N. K. Schultz in his recent article on the preparation of some nutrient media.[1] His statement that the bouillon should be neutralized before the addition of the gelatinizing substances (agar-agar or gelatine) is contrary to our experience, as we have found watery solutions of agar-agar feebly alkaline and those of gelatine strongly acid, so that it is necessary to neutralize after, instead of before, the addition of these substances. On the other hand we agree with Schultz in his recommendation of phenolphthalein as a delicate indicator of alkaline reaction, and that there are also advantages in the use of a solution of caustic soda, instead of carbonate of

[1] N. K. Schultz. Zur Frage von der Bereitung einiger Nährsubstrate. Centralbl. f. Bakteriologie und Parasitenkunde, Bd. X., p. 52.

soda, for neutralization, as thereby there is no development of carbonic acid to prevent the ready recognition of the neutral or alkaline reaction.

His experience also coincides with ours as to the relation between the reaction of the nutrient medium on the one hand and its transparency and readiness of filtration on the other hand.

That Schultz's method of making nutrient agar-agar requires much longer time than ours is evident from his statements that in his rapid method (No. II.) the boiling requires at least three hours (it is not altogether clear from his description whether it requires three hours or five hours), and that the filtration, for which he uses a hot water filter, requires about one hour. By our method, as already stated, filtration of a litre through an ordinary filter is accomplished in a few minutes, and the whole process of preparation of the medium need not take over one hour.

By the process described the filtration of the agar media is even more rapid than that of media containing gelatine, and we now regard the preparation of nutrient agar as less laborious, troublesome and time-consuming than that of nutrient gelatine.